

IN THE SPECIFICATION:

Please amend paragraph [0045] as follows:

[0045] FIGS. 18A, 18B, 18C and 18D show chemical formulas of photo-affinity crosslinkers useful in the invention and partial chemical reactions for photo-coupling the crosslinkers to a base coating; and

Please amend paragraph [0064] as follows:

[0064] In an alternate and presently preferred embodiment, light source 100 is a laser diode emitting in the red wavelength region of 600-700 nm, available from Toshiba (part no. TOLD 9211). This laser diode provides about 5 milliwatts of power with a peak emission wavelength of about 670 nm. Laser diodes emitting at 630 nm are also available and can be used. For an embodiment using wavelength in this region, it is necessary to use dyes such as cyanine dyes, whose fluorescence can be stimulated by excitation with wavelengths in the red spectral region. An example of such a dye is CY5, available from Biological Detection Systems, Inc., Pittsburgh PA (catalog no. A25000). The CY5 dye can be conjugated to the desired tracer molecule by the manufacturer's instructions and/or with a kit available from BDS. A second dye, CY7, which is available from the same source, may also be suitable. The dyes and methods for conjugating are also characterized in the paper by Southwick, P.L., et al., titled "Cyanine Dye Labeling Reagents - Carboxymethylindocyanine Succinimidyl-~~Esters~~", Esters," *Cytometry* 11:418-430 (1990). The use of laser diodes as a light source permits the biosensor and waveguide to be formed of plastic, thereby reducing the manufacturing expense and facilitating the integral molding of the semicylindrical lens with the waveguide and reservoirs.

Please amend paragraph [0071] as follows:

[0071] The design with at least two individual reservoirs has significant advantages over a single reservoir embodiment for instances in which it is desirable to measure the test sample fluorescence simultaneously with fluorescence from a control region on the same

waveguide. For example, the level of nonspecific binding to the waveguide can be subtracted from the test sample fluorescence. Also, corrections can be made for measurement changes due to fluctuations in intensity of the exciting light ~~can be corrected for~~. In a displacement assay, the “control” region could be the preloaded waveguide with no analyte present in the sample, or with a known amount of analyte. With three or more wells, fluorescence can be measured for both a no-analyte control and at least one known, calibration analyte sample in addition to the “unknown” or test sample.

Please amend paragraph [0080] as follows:

**[0080]** In a presently preferred further embodiment, the waveguide is also formed of the optical plastic and is molded simultaneously with the lens and/or the reservoirs. The latter type of construction is not suitable for use with excitation wavelengths of 488 to 515 nm, because known optical plastics tend to emit fluorescence when excited in this (the blue and green) wavelength region. This fluorescence would appear as background fluorescence. However, an alternate embodiment of the apparatus using a light source emitting at wavelengths of 600 nm and above would accommodate a plastic waveguide. Molding the lens/waveguide, or lens/waveguide/reservoir(s), as a single unit of plastic substantially reduces the cost of manufacturing and makes a disposable biosensor more feasible.

Please amend paragraph [0113] as follows:

**[0113]** The modified PEG was coupled to silanized-glutaraldehyde-treated waveguide surfaces prepared as described in Example I. A solution of 24 milligrams (mg) of PEG-ED powder was dissolved in 1.2 milliliters (ml) of 0.15M PBS, pH 7.4, or in the same volume of 11% potassium sulfate-sodium acetate buffer at pH 5.2. The prepared waveguide surfaces were immersed in the PEG-ED solution and incubated at 60°C for about 24 hours. The procedure using K<sub>2</sub>SO<sub>4</sub>-acetate buffer yielded a higher density of PEG molecules attached to the surface than that using PBS buffer. Antibodies or other binding proteins were immobilized to the

PEG-coated waveguides as follows. A solution of about 3 mg/ml of antibody was dissolved in 0.15 M sodium acetate buffer, pH 5.2. A solution of equivalent weight of 50 mM sodium metaperiodate ( $\text{NaIO}_4$ ) was then added, and the reactants were agitated at room temperature for about an hour. Unreacted sodium metaperiodate was removed by passing the reaction mixture through a desalting column (type PD-10 from Pharmacia), which had been pre-equilibrated with the sodium acetate buffer.

Please amend paragraph [0130] as follows:

[0130] For purposes of the tests shown in FIGS. 7A, 7B and 8, the antibody to be detected (the analyte) was chosen to be a monoclonal antibody (designated anti-hCG-A) to an hCG antigen (the latter designated hCG-A). The data of FIG. 7A were obtained with whole hCG molecules serving as the capture molecules (the antigen or analyte binding molecule) in the assay. The data of FIG. 7B were obtained using an oligopeptide constructed to selectively bind the anti-hCG-A antibody as the capture molecules. Oligopeptides suitable for this purpose for any known antigenic analyte molecule analyte can be obtained using the methods of Geysen et al. as disclosed in Patent Publication Nos. ~~No.~~ WO 86/86487 and U.S. Patent No. 4,708,871, as well as in the scientific literature. To attach the necessary fluorescent dye, either the N-terminus of the oligopeptide was modified to provide an amino group for amino-reactive dyes, or the C-terminus was modified to provide a cysteine thiol group for thiol-reactive dyes. Preferably also, the complete oligopeptide sequence is of length sufficient that the attached dye is spaced from the binding site by at least two or three residues.

Please amend paragraph [0134] as follows:

[0134] FIGS. 7A and 7B show the results for a sandwich assay format following the binding of anti-hCG-A to immobilized hCG and to the oligopeptide, respectively. ~~Figure~~ FIG. 8 shows the corresponding fluorescence enhancements for both cases. The data from FIGS. 7A and 7B were normalized for background fluorescence and replotted as fluorescence enhancement

( $F_{\text{sample}}/F_{\text{reference}}$ ) versus log analyte concentration. The response curve was similar for both of the immobilized antigens (whole hCG and oligopeptide antigen) over a range of antibody concentrations from  $10^{-13}$  M to  $10^{-10}$  M. However, whole hCG gave better precision.

Please amend paragraph [0143] as follows:

[0143] A presently preferred coating is a type of compound referred to herein as a “~~block copolymer~~”, copolymer,” comprising at least one hydrophilic block containing polymerized hydrophilic residues (polyethylene oxide, “PEO”) adjacent at least one hydrophobic block containing polymerized hydrophobic residues (polypropylene oxide, “PPO”). A subclass of such compounds referred to herein as “triblock copolymers” or “~~TBCPs~~”, TBCPs,” comprises a hydrophobic block flanked by hydrophilic blocks. A series of TBCPs is commercially available from BASF Corporation under the tradename PLURONICS. An example of a presently preferred compound is known generally in the literature as PLURONICS F108 or “PF108”; it has a molecular weight (MW) of about 14,600 and the general formula  $(\text{PEO})_x(\text{PPO})_y(\text{PEO})_x$ , where  $x = 129$  and  $y = 56$ . In block copolymers, the hydrophobic PPO segment tends to adsorb strongly to plastics including polystyrene, leaving the PEO side-arms in a relatively mobile state. Block copolymers have the general property of inhibiting nonspecific protein adsorption, while providing hydrophilic side chains useful to attach proteins, including Fabs or Fab fragments.

Please amend paragraph [0145] as follows:

[0145] Referring to FIG. 16, a general procedure for preparing a patterned polystyrene waveguide is as follows. First, a waveguide surface 700 coated with PF108 molecules 702 is prepared. Next, the free PEO chain ends 704 of the PF108 molecules 702 in a selected region of the waveguide are derivatized in a photo-activated coupling reaction with a photoaffinity crosslinker 706. Suitable crosslinkers are heterobifunctional reagents which have a photo-activatable group conjugated to a reactive functional group such as isothiocyanate,

succinimide or maleimide. Upon irradiation with light beam 701 of the appropriate wavelength (generally in the ultraviolet region), the photo-activatable groups of the crosslinker 706 react to covalently bind to the free PEO chain ends 704. A mask 712 (FIG. 15) confines the irradiation to a first region 714 of the waveguide. The result ~~is the~~ is a waveguide surface having reactive functional groups useful to bind Fab' fragments only in the first region 714. Next, the waveguide surface 700 is incubated with a solution of Fab' fragments of a first species (FAB 1 720 in FIG. 16) for a time sufficient to allow the binding of Fab' fragments to the derivatized region to go to completion. The unreacted Fab' fragments are then washed off, and the process of photo-activated derivatization is repeated for a second region of the waveguide, followed by incubation with a second species of Fab' fragment.

Please amend paragraph [0148] as follows:

[0148] Suitable photoaffinity crosslinkers include aryl azides (amine-to-amine linkage), fluorinated aryl azides (C-H bond-to-amine linkage), and benzophenones (C-H bond-to- amine linkage or C-H bond-to-thiol-linkage, depending on the specific compound). Examples of each type are shown in FIGS. 18A-18D, along with the corresponding photo-activated coupling reaction. Presently, benzophenones providing a C-H bond-to-thiol linkage are preferred, as these can be used to achieve site-specific coupling to Fab' fragments. Either the iodoacetamide or the maleimide derivatives of benzophenone ("BPIA" and "~~BPM~~", "BPM," respectively) can achieve this purpose. At present BPM is preferred, as it exhibits a higher degree of specific binding and a lower degree of nonspecific adsorption. This is because the coupling occurs via the C-terminal thiol groups of the Fab' fragments, as described previously herein for the PMahy coating. Other photo-affinity crosslinkers providing free maleimido groups may be equally suitable.